INTERSPECIFIC HYBRIDIZATION OF *LILIUM LONGIFLORUM* THUNB.

by

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AN IMPROVED POLLINATION TECHNIQUE FOR INTERSPECIFIC LILY CROSSES SUFFERING FROM INCOMPATIBLE POLLEN TUBE GROWTH[1]

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SUMMARY: Pre- and post- pollination applications of Easter lily stigmatic exudate on cut styles in stylar-amputation pollinations in 1977 resulted in embryo formation and, after embryo culture, in plants. Stylar-amputation pollinations made in 1976 using only pre-pollination application of stigmatic exudate gave only a limited number of embryos and no plants. Pollinations were made using *Lilium longiflorum* or 1 of 4 Aurelian hybrids as female and crossing them with various Asiatic hybrid lilies.

INTRODUCTION

Attempts to cross the Easter lily, *Lilium longiflorum* Thunb., with other lily species and hybrids seldom have succeeded due to incompatible pollen tube growth, endosperm abortion and endosperm toxins (Ascher and Drewlow 1975, Brandram and Dowrick 1969, Emsweller, Asen and Uhring 1962). Various methods have been employed to overcome these barriers. Embryo culture is widely used to prevent the death of small immature lily embryos after successful fertilizations by preventing embryo starvation due to endosperm abortion or by preventing embryo death due to toxins such as ferulic acid that are produced in the endosperm and move into the embryo

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(Ascher 1973, Emsweller, Asen and Uhring 1962, North 1970, Myodo 1975, Myodo and Asano 1977). Lily embryos removed from seeds in as little as 35 days successfully grew into seedlings (Clark and Campbell 1979).

The main barrier preventing the hybridization of many lilies is interspecific incompatibility, the failure of pollen tubes to grow through the style and reach the ovules. One method to overcome interspecific incompatibility is to maximize pollen tube growth. Pollen tubes from interspecific crosses growing 48 hr at room temperature in detached Easter lily styles are classified as compatible if they grow through 80-90 mm of 100 mm Easter lily styles, half-growth incompatible if they grow approximately 50 mm, or short-growth incompatible if they grow 10-15 mm (Ascher and Drewlow 1971, 1975).

Pollen tubes in reciprocal crosses can grow in one of the above ways or in any combination of two depending on the parental relationship. Reciprocal crosses between closely related lilies (the same botanical section, Comber 1949) usually suffer from unilateral interspecific incompatibility: Compatible pollen tube growth occurs when the cross is made one way, while in the reciprocal cross short-growth incompatible pollen tube growth occurs (Ascher and Drewlow 1975). Distantly related lilies (different botanical sections) usually suffer from reciprocal half- or short- growth incompatibility. Unilateral interspecific incompatibility is overcome by pollinating in the direction that gives compatible pollen tube growth. Reciprocal half- or short- growth interspecific incompatibility may be overcome by pollinating in the direction that gives the greatest percent growth through the style. This does not mean placing the pollen of the lily having the longest style on the lily having the shortest style. If the percentage penetration of the style by pollen tubes is

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approximately the same, the cross is made in both directions.

If reciprocal short-growth and half-growth interspecific incompatibility cannot be overcome by planned pollinations, additional measures are needed. Napthalene acetamide (NAD) has been used by Emsweller and Stuart (1948) to overcome self- and interspecific- incompatibility in Easter lilies. NAD kept seed capsules green longer whether they had viable seeds or not. This allows pollen tubes more time to grow through the style and fertilize the ovules. Also NAD keeps seed pods with only a few fertilized seeds alive longer so viable seeds can be harvested.

Stylar-amputation pollinations have been used successfully to overcome self- and interspecific- incompatibility in the genus _Lilium_ (Ascher 1977, Cheng and Mattson 1972, Myodo 1975, Myodo and Asano 1977, Sagawa 1959, Watts 1967). To make a stylar-amputation pollination on lilies, one half to all of the style is removed and the remaining stylar portion or the top of the ovary is pollinated; or pollen is packed into the hollow style through an opening in the cut surface.

Stylar-amputation pollinations require pollen to be placed on the cut stylar surface or packed into the stylar canal. Pollen grains will not germinate on or grow through dry or damaged tissue. The cut stylar surface dries quickly after amputation preventing pollen germination unless it is protected from drying. Packing pollen into the stylar canal enables pollen grains to germinate in a moist environment, but pistil packing can damage the tissue in the stylar canal preventing normal germination and growth. Also the stylar exudate in the stylar canal is not the natural medium for pollen germination. Stigmatic exudate can be used to carry pollen grains into the style past damaged tissue since it is absorbed into the style when placed on the cut stylar surface (Ascher

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1977). Once in the stylar canal pollen grains germinate in the stigmatic exudate, their normal germination medium. Stigmatic exudate has little or no effect on pollen tube growth in interspecific (Ascher and Drewlow 1975) or in intraspecific pollinations (Ascher and Drewlow 1970).

This research, done in the summers of 1976 and 1977, was designed to intercross various Asiatic hybrids, Aurelian hybrids, and Easter lily cultivars using stylar-amputation stigmatic-exudate pollinations and embryo culture. A pollination technique was discovered which greatly increased the percentage of fruit set and embryo production.

MATERIALS AND METHODS

Pollinations were made as follows. Tepals and stamens were broken off at the base of the ovary. Using a sharp, acetone-cleaned razor blade the style was cut at a 45° angle 1 cm above the ovary (cut surface up) creating a platform (Fig. 1). A drop of Easter lily stigmatic exudate was applied immediately to the platform. After approximately 2 or 3 min, pollen was dabbed generously onto the exudate and mixed with it. More exudate was added to the platform until the exudate almost ran down the style. Pollinations in 1976 differed from pollinations in 1977 in that in 1976 only a pre-pollination application of stigmatic exudate was made and in 1977 pre- and post- pollination applications of stigmatic exudate were made.

In 1977, plants grown indoors were pollinated 3 times and outdoor grown plants were pollinated twice; in 1976, indoor grown plants were pollinated 3 times and outdoor plants once. To repollinate a flower, a small portion (3mm) of the remaining style was cut off, exudate was applied to the remaining stylar portion and then pollen and another drop of exu-

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date was applied. The more exudate that can be applied to the style the better.

Stigmatic exudate was collected twice daily from the stigmas of Easter lilies using a syringe and needle and stored in glass vials in a freezer at -20° C for later use. Pollen was collected from newly opened flowers, and allowed to dry for a day on the anthers. Pollen not immediately used was placed in glass vials and frozen at -20° C for a short period of time. Stigmatic exudate can be thawed and refrozen, but pollen should not be refrozen.

Species and hybrids used in the crosses are listed in Table 1. Pollen tube studies were made before the pollinations were made (Fig. 2).

Seeds from these crosses lack endosperm so seed capsules with embryos were harvested from 30 to 70 days after pollination. The embryos were removed aseptically in a laminar flow hood and transferred to either Norstog's (1973) medium or Emsweller, Asen and Uhring's (1962) medium for growth and development.

RESULTS

Comparison of the results of the two years (Table 2), shows that pre- and post- pollination application of stigmatic exudate resulted in more capsules with embryos (179 vs. 7), more embryos (2810 vs. 45), and more plants produces (120 vs. 0) than crosses using just pre-pollination application of stigmatic exudate. The percentage of pods with embryos in 1977 ranged from 0% to 83.9% depending on the type of cross. The number of embryos found in pods with embryos averaged from 1 for the cross *Lilium* 'Pink Perfection' X *Lilium longiflorum* 'Nellie White' to 33 for the cross *Lilium longiflorum* 'Nellie White' X *Lilium* X 'Greenbush #7' x 'Greenbush #9'

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or Lilium X 'Giftbulb 66'. One *Lilium longiflorum* 'Ace' X *Lilium tigrinum* '66-12' seed capsule had 77 embryos excised from it. Thirty-seven pods have had embryos excised from them that have developed into plants. So far approximately 120 seedlings have been potted. Some plants are still developing in vials and will be planted in the future.

DISCUSSION

The difference between pollinations made in 1976 and 1977 was the amount and the number of times stigmatic exudate was applied during pollinations. Stigmatic exudate is sucked into stylar canal carrying pollen grains with it. Inside the style pollen grains are not subject to drying and can germinate normally. When more stigmatic exudate is applied during pollinations more pollen is carried into the stylar canal and there is a greater chance of fertilization. We knew when enough stigmatic exudate was applied during the previous pollination, if examination of the recut style during repollination showed pollen grains in the stylar canal.

Other researchers have obtained successful pollinations in interspecific lily crosses using stylar amputation pollinations without stigmatic exudate (Cheng and Mattson 1972, Myodo 1975, Myodo and Asano 1977, Sagawa 1959, Watts 1967). Pollen grains in these pollinations were packed into the stylar canal with a flexible fiber or the style was split open longitudinally and pollen placed in the canal. These techniques can damage the stylar canal and prevent normal pollen tube germination and growth. Our technique differed from these methods in that the style was cut off so that a platform was made and stigmatic exudate was applied to the platform to carry pollen into the style instead of packing pollen into the style with a flexible fiber.

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Stylar- amputation stigmatic-exudate pollinations are easier to do than other stylar-amputation techniques and give as good or better results than other methods. Cheng and Mattson (1972) obtained from 0 to 13.4 seeds per capsule after selfing self-incompatible 'Mid-Century hybrid' lilies using stylar-amputation. Myodo (1975) and Myodo and Asano (1977) have obtained numerous hybrid lilies between Asiatics and Aurelians, between Asiatics and Trumpets and between Orientals and Trumpets using stylar-amputation. They obtained between 3.3 and 93.8% pods with embryos and from an average of 1 to 84.1 embryos per pod depending on the cross. Sagawa (1959) got only a limited number of seeds from self pollinations using stylar amputation. Watts (1967) got normal seed set using stylar-amputation pollinations in crosses that normally are successful. He also obtained increased seed set from a pseudo-self-incompatible lily cross using stylar amputation.

It is apparent from the above data that stylar-amputation pollinations can be used to cross distantly related lilies which suffer from incompatible pollen tube growth. Stigmatic exudate shows promise in increasing seed set and embryo formation in stylar-amputation pollinations.

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Figure 1. Diagram of an Easter lily flower after tepals and stamens have been removed and the style amputated. Note platform made by cutting the style at a 45° angle.

Figure 2. Pollen tube lengths in lily styles after 48 hr at room temperature. Solid lines indicate pollen tube lengths, dashed lines indicate style lengths, S= Style, SE= Stigmatic exudate, MINUS STG= Stigma removed, $\frac{1}{2}$ S= $\frac{1}{2}$ Style removed, $\frac{1}{4}$ S = $\frac{1}{4}$ Style removed.

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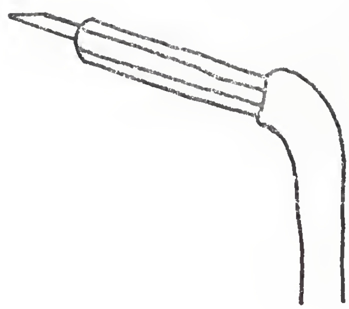

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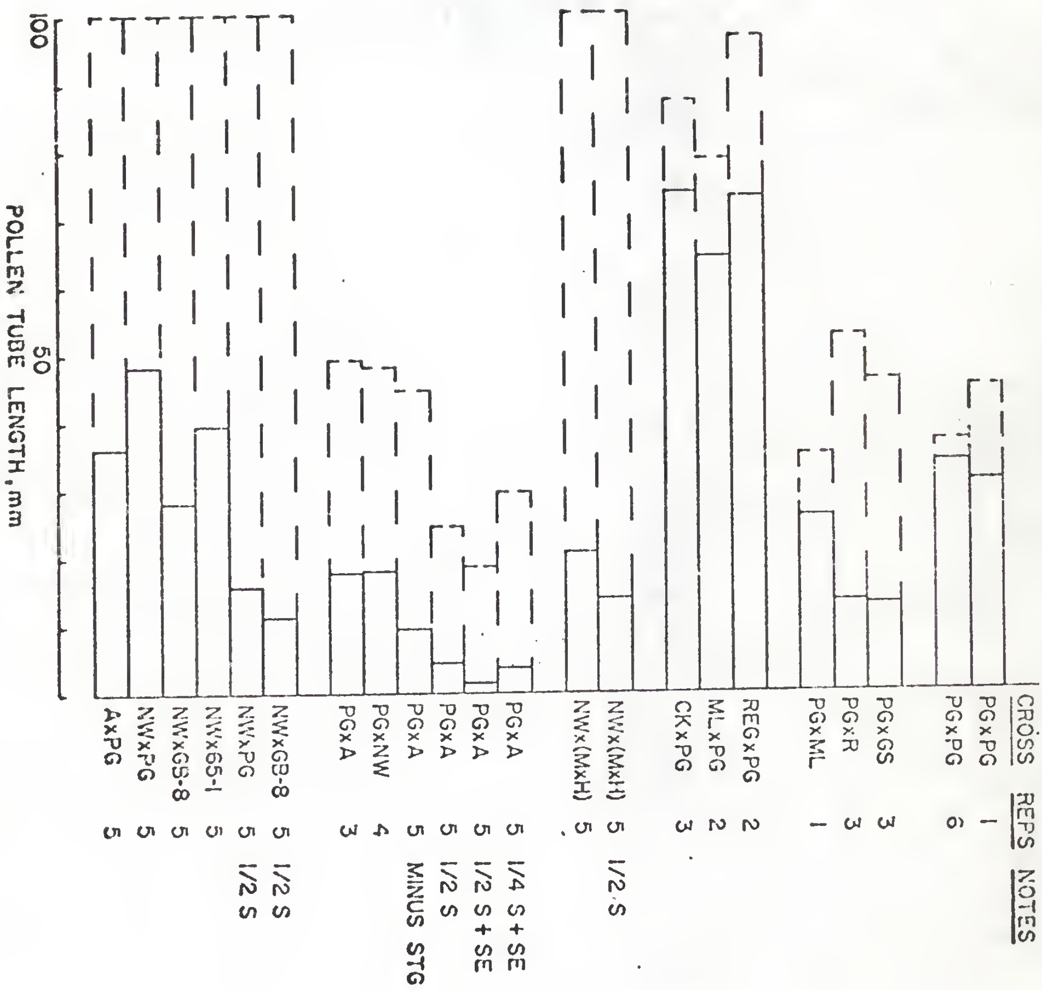
100
50
POLLEN TUBE LENGTH, mm
CROSS REPS NOTES
PGxPG 1
PGxPG 6
PGxGS 3
PGxR 3
PGxML 1
REGxPG 2
MLxPG 2
CKxPG 3
NWx(MxH) 5 1/2 S
NWx(MxH) 5
PGxA 5 1/4 S + SE
PGxA 5 1/2 S + SE
PGxA 5 1/2 S
PGxA 5 MINUS STG
PGxNW 4
PGxA 3
NWxGB-8 5 1/2 S
NWxPG 5 1/2 S
NWx65-1 5
NWxGB-8 5
NWxPG 5
AxPG 5

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TABLE 1. List of the lilies used and the crosses made each year.

Female Parent		Male Parent	Abbreviation	Year
Lilium longiflorum 'Nellie White'	X	Mid-Century hybrid 'Painters Glow'	NW X PG	76 77 78
" " 'Ace'	X	" " " "	A X PG	76 77 78
Aurelian hybrid 'Moonlight'	X	" " " "	ML X PG	76 77
" " 'Copper King'	X	" " " "	CK X PG	76 77
" " 'Golden Sunburst'	X	" " " "	GS X PG	76 77
" " 'Regal'	X	" " " "	R X PG	76 77
Lilium longiflorum 'Nellie White'	X	Rainbow hybrid '65-1'	NW X 65-1	77
" " 'Ace	X	" " "	A X 65-1	77
" " 'Nellie White'	X	" " '66-2'	NW X 66-2	77
" " 'Ace'	X	" " "	A X 66-2	77
" " 'Nellie White'	X	Greenbush hybrid 'Early Red Cup #8'	NW X GB#8	77
" " 'Ace'	X	" " " " " "	A X GB#8	77
" " 'Nellie White'	X	" " 'Yellow Cup #9'	NW X YC#9	77
" " " "	X	" " '67-1'	NW X 67-1	77
" " " "	X	'Greenbush hybrid '#7 x Greenbush hybrid #9'	NW X 7x9	77
" " " "	X	Mid-Century hybrid 'Harmony'	NW X Har	77
" " 'Ace'	X	" " "	A X Har	77
" " 'Nellie White'	X	'Mountaineer'	NW X M	77
" " 'Ace'	X	'Mountaineer'	A X M	77
" " 'Nellie White'	X	'Talisman'	NW X Tal	77
" " 'Ace'	X	"	A X Tal	77
" " 'Nellie White'	X	*Lilium tigrinum* open pollinated '66-12'	NW X 66-12	77
" " 'Ace'	X	" " " " "	A X 66-12	77
" " 'Nellie White'	X	*Lilium martagon* x *L. hansonii*	NW X MxH	77
" " " "	X	'Gift Bulb 66'	NW X G66	77
'Pink Perfection'	X	Lilium longiflorum 'Nellie White'	PP X NW	77
" "	X	" " 'Ace'	PP X A	77
'Mrs. Bangs hybrid'	X	" " 'Nellie White'	Mrs B X NW	77
" " "	X	" " 'Ace'	Mrs B X A	77
'Yellow Trumpet #3 x Yellow Trumpet #1'	X	" " 'Nellie White'	T3xT1 X NW	77
" " " " " " "	X	" " 'Ace'	T3xT1 X A	77
'Mrs. Bangs hybrid x Yellow Trumpet'	X	" " 'Nellie White'	66-10 X NW	77
'Gift Bulb 65 x Stiff Centifolium'	X	" " " "	66-9 X NW	77
Lilium henryi selfed x Gift Bulb 65	X	" " " "	66-8 X NW	77
" " " " " " "	X	" " 'Ace'	66-8 X A	77
'Orange Star Gift Bulb 61 x Yellow Trumpet'	X	" " "	61xYT X A	77
'Down-facing Yellow Trumpet x Outfacing Yellow Trumpet'	X	" " 'Nellie White'	DYTxOYT X NW	77
'Down-facing Yellow Trumpet x Outfacing Yellow Trumpet'	X	" " 'Ace'	DYTxOYT X A	77

TABLE 2. Results of the stylar-amputation stigmatic-exudate pollinations made in 1976, 1977 and 1978. 1977 and 1978 pollinations are also listed by type of cross.

YEAR	CROSS	NO. OF FLOWERS POLL.	NO. OF POLL. MADE	PODS WITH EMBRYOS	% PODS WITH EMBRYOS	NO. OF EMBRYOS	NO. OF EMBRYOS PER POD	% EMBRYOS PER POD	NO. OF PLANTS	NO. OF PODS WITH PLANTS
1976		2670	5736	7	.26	45	15.2	2.66	0	0
1977		971	2670	179	18.4	2810	15.2	5.66	120	37
	R X PG	2		1	50.0	15	15.0	5.0	0	0
	GS X PG	8		2	25.0	18	9.0	3.0	0	0
	ML X PG	2		1	50.0	6	6.0	2.0	0	0
	NW X PG	51		10	19.6	176	17.6	5.9	15	3
	A X PG	36		3	8.3	15	5.0	1.7	8	2
	NW X 65-1	48		11	22.9	128	11.6	3.9	5	4
	A X 65-1	25		2	8.0	18	9.0	3.0	0	0
	NW X 66-2	49		4	8.2	47	11.8	3.9	0	0
	A X 66-2	31		12	38.7	236	19.7	6.6	10	4
	NW X GB#8	48		4	8.3	38	9.5	3.2	0	0
	A X GB#8	29		3	10.3	14	4.7	1.6	0	0
	NW X YC#9	50		11	22.0	77	7.0	2.3	2	1
	NW X 7x9	69		13	18.8	127	9.8	3.3	4	3
	NW X 7x9 or X G66	17		1	5.9	33	33.0	11.0	0	0
	NW X G66	26		2	7.7	21	10.5	3.5	0	0
	NW X 67-1	97		9	9.3	89	9.9	3.3	1	1
	NW X Tal	44		6	13.6	68	11.3	3.8	3	1
	A X Tal	35		20	57.1	356	17.8	5.9	12	6
	NW X 66-12	50		29	58.0	489	16.8	5.6	12	4
	A X 66-12	31		26	83.9	751	28.9	9.6	12	6
	A X Har	33		5	15.2	28	5.6	1.9	0	0
	PP X NW	39		1	2.6	1	1.0	.3	0	0
	PP X A	41		4	9.8	59	14.8	4.9	29	2
	Others	110		0	0.0	0	0.0	0.0	0	0
1978		52	156	0	0.0	0	0.0	0.0	0	0
	NW X PG	30	90	0	0.0	0	0.0	0.0	0	0
	A X PG	22	66	0	0.0	0	0.0	0.0	0	0

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INTERSPECIFIC HYBRIDIZATION OF LILIUM LONGIFLORUM THUNB. USING STYLAR-AMPUTATION STIGMATIC-EXUDATE POLLINATIONS AND EMBRYO CULTURE[1]

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INDEX WORDS

Lilium longiflorum, Easter lily, interspecific incompatibility, stigmatic exudate, stylar amputation, embryo culture, pollen tube growth.

SUMMARY

Lilium longiflorum Thunb., the Easter lily, was crossed with numerous Asiatic hybrid lilies and Aurelian hybrid lilies. The pollination procedure involved cutting off at a 45^{o} slant all but 1 cm of the style, applying Easter lily stigmatic exudate to the cut style surface, mixing pollen with the stigmatic exudate and reapplying stigmatic exudate. Flowers were repollinated twice by cutting off 3 mm of the remaining style, applying stigmatic exudate to the remaining stylar portion, and applying pollen and another drop of stigmatic exudate. Stigmatic exudate carried pollen grains into the stylar canal. Embryos were excised 30 to 70 days after pollination and ranged from small globular embryos to large torpedo shaped embryos. Approximately 120 hybrids with Lilium longiflorum have been obtained using these techniques.

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INTRODUCTION

The Easter lily, Lilium longiflorum Thunb., is the traditional flower for Easter in the United States and usually is forced only for the Easter season. The large white flowers; dark green, closely spaced foliage; compact size; and ease and predictability for year round forcing make it an excellent greenhouse pot plant. In Europe Easter lilies and many Trumpet, Asiatic and Oriental lilies are sold year round as cut flowers. Growers gladly would force the Easter lily year round if the American public could be convinced to buy the Easter lily year round, perhaps through additional flower colors. There are many colored trumpet lilies with a flower shape similar to the Easter lily available to the growers, but they are unpredictable in forcing, have pale green, widely spaced foliage and are tall and top heavy. These colored lilies are suitable only for cut flower production. There is a need to develop colored Easter lilies for pot plant culture.

The Easter lily is a poor, short-lived garden plant, but it has many desirable garden characteristics such as compact size; long, wide, dark green foliage; and large showy flowers. Few garden lilies have all of these traits. Most Trumpet lilies are too tall and visually top heavy. Most Asiatics, although wonderful garden plants, lack trumpet shaped flowers. There is a need to incorporate these desirable traits of the Easter lily into the more hardy garden lilies.

The genus Lilium is divided into 7 botanical sections (Comber 1949) containing 80 to 90 species and 9 horticultural divisions (Anonymous 1964) which contain all of the hybrid lilies as well as all species. The genus has a wide range of flower shapes, colors and sizes. Foliage varies in color, size, density and in manner of growth, whether whorled or scattered.

Plant height ranges from 30 cm dwarfs, to giants over 2.4 m. Lilies vary in their drought tolerance, disease and insect resistance, and cold hardiness. If it were possible to intercross all lily species and hybrids there would be an almost unlimited number of hybrid forms available. However, crosses between lilies of different botanical sections and many times even within the same section are rare, although they do occur. Attempts to hybridize the Easter lily with other lily species and hybrids have been unsuccessful. There were 2 reported Easter lily hybrids using normal pollination procedures, both L. formosanum X L. longiflorum (Emsweller 1955, Tieman 1969). Emsweller has argued the progeny from 1 of these crosses were not true hybrids but L. formosanum seedlings.

Many factors contribute to the inability of the Easter lily to cross. Some lily species or hybrids when crossed produce male and or female sterile hybrids (Ronald and Ascher 1976). Hybrid sterility can be overcome in some cases by crossing with a bridging species (Ascher 1969), by making massive pollinations, by backcrossing if the hybrid is either male or female sterile or by doubling the chromosome number.

Failure of the seed to develop properly after fertilization is another serious problem in lily breeding. Many times the endosperm fails to develop or develops abnormally (Emsweller, Asen and Uhring 1962, Myodo 1975, Myodo and Asano 1977, and North 1970). Lack of double fertilization may be a cause since in any one pod some seeds have normal endosperm development but no embryos and other seeds have no endosperm but have embryos (Ronald and Ascher 1975, Stout and Porterfield 1938). Also, abnormal mitotic chromosome division in the pentaploid lily endosperm can cause the endosperm to develop abnormally or die (Brandram and Dowrick 1969). These embryo-endosperm problems can be overcome by removing the embryo from the

seed before the seed capsule turns brown and growing the embryos on an artificial nutrient medium (Ascher 1973, Emsweller, Asen and Uhring 1962, Myodo 1975, Myodo and Asano 1977, North 1970, Ronald and Ascher 1976). A study of the types of culture medium and their ability to grow lily embryos to the seedling state was made by Stimart and Ascher (1974). They found that the medium developed by Emsweller, Asen and Uhring (1962) worked best for large, nearly-mature, torpedo-shaped embryos and Norstog's (1973) medium worked best for small, globular, immature embryos.

Interspecific incompatibility is the main barrier to successful crosses between the Easter lily and other lily species and hybrids. It is the failure of the pollen tubes to grow through the style into the ovary and fertilize the ovules. Pollen tubes growing in Easter lily styles after interspecific crossing are classified as compatible-growth pollen tubes if they grow 80 to 90 mm through the 100 mm Easter lily in 48 hr at 24° C; half-growth incompatible if they grow 30-50 mm, similar to self-incompatible Easter lily pollen tubes; or short-growth incompatible if they grow 5-15 mm (Ascher and Drewlow 1970, 1975). Evidence suggests that half-growth incompatibility is due to the inability of the foreign pollen to use the food secreted by the cells lining the stylar canal (Ascher and Peloquin 1968). Short-growth incompatible pollen tube growth may result from the inhibition of the pollen tubes as they enter the stylar canal from the stigma as these pollen tubes appear abnormal in morphology (Ascher and Peloquin 1968).

Unilateral interspecific incompatibility between 2 species involves short-growth and compatible type pollen tube growth rates depending on which direction the crosses is made. As an example, when _L. longiflorum_ is crossed as male with closely related trumpet lilies like _L._ X 'Damson',

compatible pollen tube growth occurs; in the reciprocal cross short-growth pollen tube growth results (Ascher 1973). Reciprocal interspecific incompatibility occurs when pollen tubes grow approximately the same lengths in reciprocal crosses involving the 2 species or hybrids. *L. longiflorum* crossed with *L.* 'Mid-Century Hybrids' usually show reciprocal half-growth incompatibility, while *L. longiflorum* X *L. henryi* and the reciprocal cross show reciprocal short-growth incompatibility. Crosses made by Ascher and Drewlow (1975) show that distantly related species or hybrids (different botanical section) usually suffer from reciprocal incompatibility, while more closely related species and hybrids (same botanical section) suffer from unilateral interspecific incompatibility.

In unsuccessful lily crosses, the type of incompatibility can be determined by studying pollen tube growth in detached lily styles as accurately as styles left on the flower (Ascher and Peloquin 1966). Once determined by pollen tube studies, unilateral interspecific incompatibility can be easily overcome by pollinating in the direction that gives compatible pollen tube growth (Ascher 1973). However, in half-growth reciprocal and short-growth reciprocal pollinations made in the direction that gives the longest pollen tube lengths as indicated by pollen tube studies, the pollen tubes will still fail to reach the ovules before floral senescence.

Two methods have been used in the past to enable incompatible pollen tubes to grow through the style and into the ovary. Emsweller and Stuart (1948) applied auxin growth hormones to the base of the ovary to overcome self incompatibility in Easter lilies. Auxins kept ovules alive longer allowing pollen tubes more time to grow down to and fertilize the ovules. Plant hormones also maintained the life of seed capsules containing only a few developing seeds. These seeds can be recovered at capsule dehiscence

or the embryos can be excised for embryo culture.

A number of researchers have used stylar amputation to overcome reciprocal interspecific incompatibility (Ascher 1977, Cheng and Mattson 1972, Myodo 1975, Myodo and Asano 1977, Nizeki 1961, Sagawa 1959, Watts 1967). These procedures involve removing from $\frac{1}{2}$ to all of the style and pollinating the remaining stylar portion or the top of the ovary, or packing pollen into the stylar canal with a flexible fiber (Table 1).

Knowing the barriers to interspecific hybridization and the methods to overcome them, a series of normally unsuccessful crosses involving L. longiflorum, 4 Aurelian hybrids, a number of Asiatic hybrids, and various colored trumpet lilies were made in the summers of 1976 and 1977 and in the winter of 1978 to try and develop new previously unobtainable, colored Easter lily hybrids as well as other hybrids.

MATERIALS AND METHODS

Lilies used and the crosses made are listed in Table 2. The Easter lily cultivars Ace and Nellie White were obtained from United Bulb Company. The Aurelian hybrids 'Moonlight', 'Regal', 'Golden Sunburst' and 'Copper King' were obtained from Oregon Bulb Farm. The Mid-Century hybrid 'Painters Glow' came from the Kansas State University Horticulture Research Farm. All other lilies used in the research were from the garden of Elizabeth (Mrs. Reginald) Painter, Manhattan, Kansas. Pollen tube studies were made to determine which direction to make the crosses (Figure 1). When in doubt, the crosses were made both ways.

Pollinations were made in the following manner: Tepals and stamens were broken off at the base of the ovary. Using a sharp, acetone-cleaned razor blade, a slanting cut at a 45° angle was made across the style 1 cm

above the ovary (Figure 2). The cut surface faced upward. A drop of Easter lily stigmatic exudate was applied immediately to the cut stylar surface. After 2 or 3 min, pollen was dabbed generously onto the exudate and mixed with it. More exudate was added to the cut end of the style until the stigmatic exudate almost ran down the style. The second stigmatic exudate application was made only in the 1977 and 1978 pollinations. Flowers on indoor grown plants were pollinated 3 times in 1976, 1977 and 1978 and flowers on outdoor grown plants were pollinated once in 1976 and twice in 1977. To repollinate a flower, a small portion of the remaining style (3mm) was cut off, stigmatic exudate was applied to the remaining stylar portion and pollen and another drop of exudate was applied.

Stigmatic exudate was collected twice daily using a syringe and needle to suck it up from the Easter lily stigma and stored indefinitely in glass vials at -20^{o} C for later use. Pollen was also collected daily from newly opened flowers and allowed to dry in the air for a day on the anthers. Pollen not used immediately was stored in small glass vials in a dessicator at -20^{o} C for a short period for later use. Stigmatic exudate was thawed and refrozen several times while pollen was frozen and thawed only once.

Pollinations were made indoors or ourdoors from April to July in 1976 and 1977 and indoors in February in 1978. Outside temperatures ranged from -2^{o} C to 35^{o} C in 1976 and from 4^{o} C to 36^{o} C in 1977. Greenhouse temperatures in February 1978 ranged from 16^{o} C to 35^{o} C.

Seeds from these crosses lacked an endosperm or had an abnormal soft endosperm, so capsules with embryos were harvested from 30 to 70 days after pollination before they dehisced if possible. Capsules with embryos turned upward after pollination and swelled. Bumps appeared on the seed pod indi-

cating where embryos were located.

The outsides of the capsules, and the insides if they had dehisced, were sterilized in a 1:10 chlorine bleach and water solution. Pods were submerged in the chlorine bleach, placed in a vaccuum dessicator for 15 sec and then left submerged in the chlorine bleach solution for 1 hr. Seed pods were slit open along suture line. The seeds were removed from the pods, floated in distilled water and examined under a dissecting microscope for embryos. Two half-shield-shaped scapels with 1 sharp edge were used to open the seeds and transfer the embryos to sterile culture medium in 8 dram vials (3 per vial) which had the mouth flamed before and after embryo transfer. Workers in the hood wore face masks and sterilized their hands, arms and tools before and after each embryo transfer with 95% alcohol. All articles put in the hood were surface sterilized.

Embryo culture media were Emsweller's medium (Emsweller, Asen and Uhring, 1962), a simple medium with major nutrients modified 2 ways: 0.6% agar and no ferric sulfate and Norstog's (1973) medium containing major, minor and trace elements as well as growth regulators, vitamins and amino acids. Emsweller's medium, prepared by dissolving the recommended chemicals in distilled water one at a time, was autoclaved for 20 min at 131° C at 20 psi, and 15 ml was poured into 8 dram vials that were then covered with aluminum foil. Norstog's medium was made as follows; we added 6 g of agar to 800 ml of deionized distilled water and autoclaved it for 15 min at 131° C at 20 psi. The other chemicals were mixed with sufficient deionized distilled water to yield 200 ml of solution, and the pH was adjusted to 4.9. The solution was then filtered through a millipore membrane (.22 um), mixed with the autoclaved component and poured into 8 dram vials that were then covered with aluminum foil.

Small, globular embryos were placed in Norstog's medium; mature, torpedo-shaped embryos were placed on either Norstog's or Emsweller's medium. After the embryos were transferred, the vials were sealed with parafilm and stored in an incubator at 27° C in the dark.

Embryos were examined weekly for growth, contamination and dessication. When embryos developed a small bulb and 1 or 2 very small leaves, they were transferred to shelves under 2 cool-white and 2 warm-white fluorescent lights (18 hr days) spaced 10 cm apart, 6-8 cm above the plants. Contaminated vials were discarded. Vials with dessicated medium had sterile water added to them with a syringe through the foil. Many times callus developed in the vials. These vials were left in the dark until bulbs appeared on the callus tissue and then the vials were placed under lights.

When the bulbs growing under lights had 2 or 3, 2-5 cm leaves and several roots, they were taken out of the vials and planted in 7.6 cm plastic or clay pots filled with coarse grade horticultural vermiculite. To acclimate them, potted seedlings were placed in plastic bags under lights for several days after which the bags were removed. They were fertilized at every watering with 100 ppm KNO_3 fertilizer. Phosphorus was added to the 100 ppm fertilizer solution every 5^{th} watering. A dilute micro-nutrient fertilizer was also used about every 2 months.

After about 4 months when the potted seedlings quit growing, miniature Christmas tree incandescent lights were added to the fluorescent lights to overcome dormancy by night interruption (Wilkins, Waters and Widmer 1968).

Seedlings were transplanted to a 1:2:2 soil, peat, perlite soil medium when the bulbs were 0.6 cm in diameter, and were transferred to a green-

house. An incandescent light was hung above these to provide night interruption. Greenhouse lilies were watered with a dilute soluble fertilizer solution at every watering.

RESULTS

Percent pods with embryos in 1977 was 18.4 % (179 pods set out of 971 pollinations) as compared to 0.26% pods with embryos (7 pods out of 2670 pollinations) in 1976: and 0% pods with embryos in 1978 (Table 3). In 1977, 2810 embryos were excised, 45 in 1976 and 0 in 1978. An average of 15.2 embryos were found in each pod with embryos in 1977, and 6.2 embryos per pod in 1976. Embryos from Easter lily seed capsules were found either in green to whitish swollen seeds which may or may not have had endosperm development, in flat brown seeds with no endosperm where embryos appeared as small bumps on the seed, or in small, thick- brown or white seeds. In 'Pink Perfection' pods, embryos, all torpedo-shaped, came from large plump green seeds with a hard or sometimes fleshy endosperm. All seed capsules in 1978 pollinations stayed green and firm for approximately 30 days then turned brown and withered. When these pods were examined between 30 and 35 days after pollinations, the first 10-20 seeds at the top of the pod usually were small, plump, clear and without embryos. Below this the seeds were not as plump and many times were brown.

Embryos which developed into plants were excised from seeds 35-69 days after pollination in 1977. The average time from pollination to excision was 52.5 days. Embryos from Easter lily seed capsules which formed plants took approximately 48.2 days from pollination to excision. Embryos from 'Pink Perfection' seed pods on the average were excised 65.7 days after pollination (2 pods). Plants were obtained from 'Pink Perfection' hybrid embryos grown on both Emsweller's and Norstog's media.

These were the only plants which developed on Emsweller's medium. Seedlings were transplanted from vials to pots an average of 98.5 days after excision with the range being from 29 to 272 days. Seedlings from L. longiflorum capsules were planted approximately 113.5 days after excision, while seedlings from 'Pink Perfection' seed pods averaged 52.5 days from excision to planting. Forty seedlings were transferred from vermiculite to soil an average of 167.6 days after planting. Embryos which did not or have not yet formed plants either died, formed calluses, were contaminated or remained dormant in the medium. Bulbs and seedlings are still developing from callus tissue and seedlings will be planted from these callus cultures in the future.

All plants have L. longiflorum as the female parent except for 29 hybrids involving 'Pink Perfection' as the female and Easter lily as male.

DISCUSSION

Lily pollen needs a moist environment for germination and will not germinate on or grow through damaged or dead tissue. In stylar-amputation pollinations the stigma, the natural site for pollen germination, is removed. Placing pollen on the cut stylar surface will result in little or no pollen germination and growth. Sagawa (1959) got a few seeds from selfing Easter lilies by cutting off all but 2 cm of the style and placing pollen on the cut stylar surface. Therefore, an artificial method must be developed for pollen germination on or in the style.

Lily breeders in the past have placed pollen in the stylar canal where it will germinate in the moist stylar exudate. Watts (1975) pushed pollen into the stylar canal down to the base of the ovary using a flexible fiber. He got increased seed set (but not full pods) using stylar-amputation pollinations on pseudo-compatible selfs and got normal seed set on

compatible interspecific crosses when compared to normal stigmatic pollinations. He also obtained seeds from the normally unsuccessful cross 'Cinnabar' X 'Golden Chalice' using stylar amputation and pistil-ramming pollinations. Myodo (1975), and Myodo and Asano (1977) have obtained numerous hybrids from interspecific crosses by cutting off all but 1 cm of the style, splitting the remaining stylar portion, placing pollen into the stylar canal and then taping the style shut with vinyl tape. Myodo got from 3.3% to 95% seed capsules with embryos and from an average of 1 to an average of 84 seeds per pod depending on the cross and the closeness of the relationship of the parents. He has bred hybrids between _L. longiflorum_ and Asiatics, between _L. longiflorum_ and Trumpets, and between Orientals and Asiatics. Cheng and Mattson (1972) got seed set from incompatible selfs and compatible crosses between 'Mid-Century hybrids' by removing all but 1 cm of t e style and placing pollen in the stylar canal or by splitting the style halfway down and packing pollen into the stylar canal.

All of the above methods require pollen to germinate in stylar exudate which is not the natural medium for germination. Also the above techniques require the pollen to be packed or rammed into the stylar canal which can damage the stylar tissue, or the pollen is left on the top of the cut style where the pollen will not germinate. Stigmatic exudate, the natural medium for pollen germination, is absorbed into the style when placed on the cut stylar surface (Ascher 1977) and can be used to carry pollen grains into the stylar canal so that the only tissue that is damaged is the cut stylar surface and not the tissue in the stylar canal. Pollen tube growth is not adversely affected by the presence of stigmatic exudate, since stigmatic exudate injected into Easter lily styles before or up to 12 hr after pollination increases pollen tube lengths in incompatible

intra-cultivar pollinations and compatible inter-cultivar pollinations (Ascher and Drewlow 1970), and in interspecific crosses which suffer from unilateral interspecific incompatibility (Ascher and Drewlow 1975). Stigmatic exudate has little or no effect on pollen tube growth in reciprocal half-growth or short-growth interspecific incompatible crosses involving _L. longiflorum_ (Ascher and Drewlow 1975).

The number of capsules with seeds and the number of embryos per pod obtained in this study are similar to or better than results obtained from other stylar-amputation pollination techniques. Also stylar-amputation stigmatic-exudate pollinations are quicker and easier to perform than other stylar amputation techniques.

Temperature or light or a combination of both have a great effect on the success of wide interspecific lily crosses. Ronald and Ascher (1976) found that L. X 'Black Beauty' when pollinated in September or February failed to produce embryos or had very small rudimentary embryos, while the same pollinations made in July were successful in producing embryos and plants. Embryos were formed in pollinations made here in Manhattan in the summers of 1976 and 1977, but not in the winter of 1978. Pollinations made in 1978 induced seed pod retention but not embryo formation. These capsules stayed green for about 30 days then turned brown and withered, while seed pods with embryos from 1977 pollinations swelled and turned upward a week or two after pollination and stayed green and firm up to 60 days. Some embryos were also found in 1977 in small pods which were similar in size and shape to 1978 seed capsules except that they turned upward.

Wide interspecific crosses are very hard to obtain and when they are

obtained you get very few plants. Any measures which can be used to increase embryo formation or to get unobtainable crosses should be investigated. Stigmatic exudate holds promise for increasing the number of embryos formed in these crosses by promoting pollen germination and growth.

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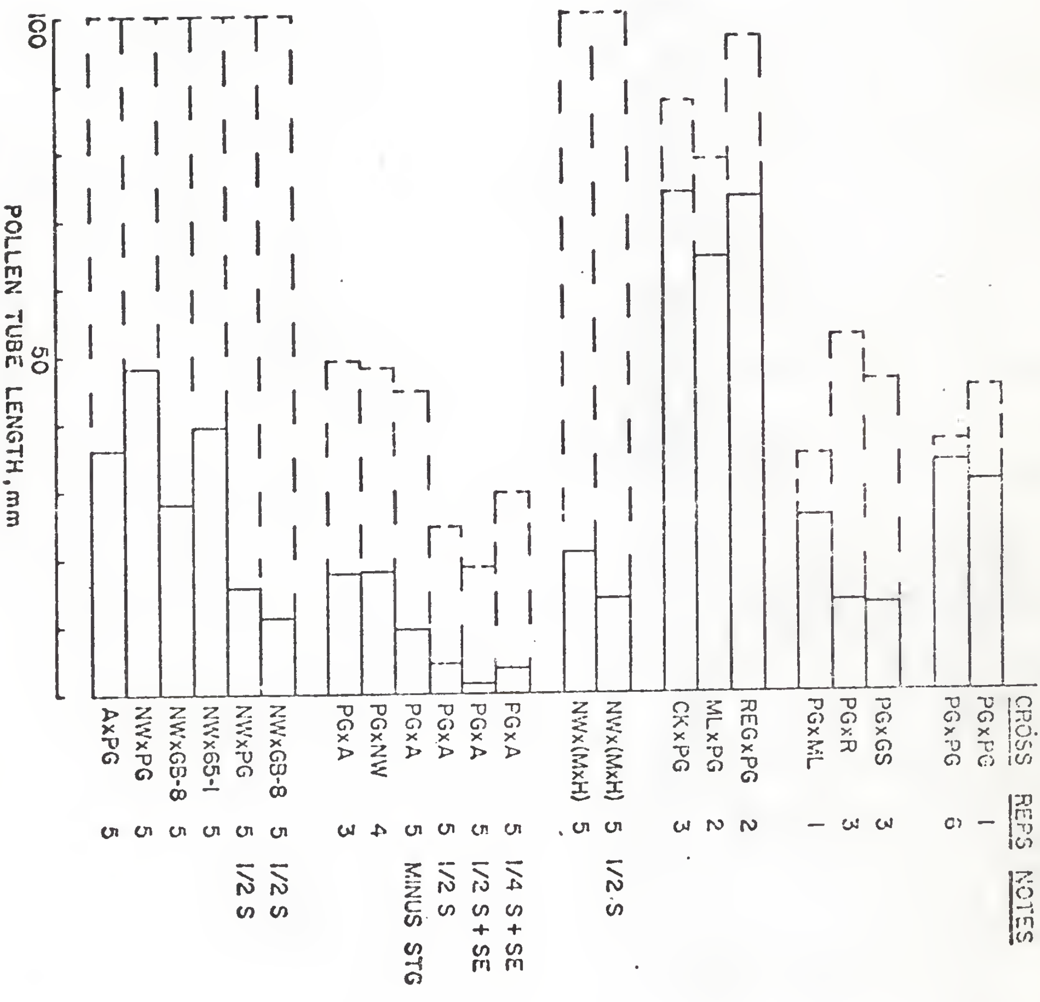
Figure 1. Pollen tube lengths in lily styles after 48 hr at room temperature. Solid lines indicate pollen tube lengths, dashed lines indicate style lengths, S= Style, SE= Stigmatic exudate, MINUS STG= Stigma removed, $\frac{1}{2}$ S= $\frac{1}{2}$ Style removed, $\frac{1}{4}$ S= $\frac{1}{4}$ Style removed.

Figure 2. Diagram of an Easter lily flower after tepals and stamens have been removed and the style amputated. Note platform made by cutting the style at a 45^{o} angle.


POLLEN TUBE LENGTH, mm
100
50
CROSS REPS NOTES
PGxPG 1
PGxPG 6
PGxGS 3
PGxR 3
PGxML 1
REGxPG 2
MLxPG 2
CKxPG 3
NWx(MxH) 5 1/2 S
NWx(MxH) 5
FGxA 5 1/4 S+SE
PGxA 5 1/2 S+SE
PGxA 5 1/2 S
PGxA 5 MINUS STG
PGxNW 4
PGxA 3
NWxGB-8 5 1/2 S
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NWxGB-8 5
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AxPG 5

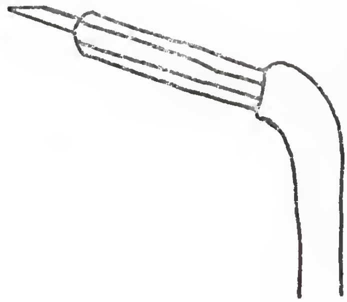

TABLE 1. List of researchers, their stylar amputation pollination technique and their results.

Author	Type of cross	C	P	I	Stylar amputation Method	Stylar pollination method	Results
Watts 1969	selfs		X		½ of style removed, cut at slant	packed pollen into style using flexible fiber, covered with foil	increased seed set on selfing. incompatible lilies, some seed set on compatible crosses
	interspecific	X		X			
Sagawa 1959	selfs			X	cut style off 2 cm above ovary	pollen placed on cut surface	got a few seeds from L. longiflorum selfs
Myodo 1975, Myodo and Asano 1977	interspecific			X	style was cut off 1 cm above ovary	remaining stylar portion split longitudinally, pollen applied to stylar canal surface, taped shut	has obtained numerous hybrids between distantly related lilies
Niizeki 1961	selfs			X	removed stigma or various lengths of the style	pollen placed on a thin sucrose agar medium on cut style, tip of style covered with gelatin and vinyl bag	pollen tubes from interspecific crosses grew through the style, self-incompatible L. tigrinum pollen tubes grew 10-15 mm
	interspecific	X					
Cheng and Mattson 1971	selfs			X	style cut off 1 cm above ovary or split halfway down style	pollen packed into stylar canal opening	got increased seed set in self incompatible selfs and reduced seed set in compatible crosses
	crosses	X					
Ascher 1977	interspecific			X	style snapped of at ovary or approx. 1 cm of the style left on	stigmatic exudate applied to top of ovary or to cut style, pollen applied to exudate, pollinated 3 or 4 times	has obtained numerous interspecific hybrids with L. longiflorum
Clark	interspecific			X	1st pollination style cut off 1 cm above ovary; 2nd and 3rd pol., 3mm more of style removed	stigmatic exudate applied to cut stylar surface, pollen mixed with exudate, exudate reapplied, pollinated 2 or 3 times	have obtained so far 120 hybrids between L. longiflorum and other lilies

C= Compatible, P= pseudocompatible, I= incompatible

TABLE 2. List of the lilies used and the crosses made each year.

Female Parent		Male Parent	Abbreviation	Year
Lilium longiflorum 'Nellie White'	X	Mid-Century hybrid 'Painters Glow'	NW X PG	76 77 78
" " 'Ace'	X	" " " "	A X PG	76 77 78
Aurelian hybrid 'Moonlight'	X	" " " "	ML X PG	76 77
" " 'Copper King'	X	" " " "	CK X PG	76 77
" " 'Golden Sunburst'	X	" " " "	GS X PG	76 77
" " 'Regal'	X	" " " "	R X PG	76 77
Lilium longiflorum 'Nellie White'	X	Rainbow hybrid '65-1'	NW X 65-1	77
" " 'Ace	X	" " "	A X 65-1	77
" " 'Nellie White'	X	" " '66-2'	NW X 66-2	77
" " 'Ace'	X	" " "	A X 66-2	77
" " 'Nellie White'	X	Greenbush hybrid 'Early Red Cup #8'	NW X GB#8	77
" " 'Ace'	X	" " " " " "	A X GB#8	77
" " 'Nellie White'	X	" " 'Yellow Cup #9'	NW X YC#9	77
" " " "	X	" " '67-1'	NW X 67-1	77
" " " "	X	'Greenbush hybrid '#7 x Greenbush hybrid #9'	NW X 7x9	77
" " " "	X	Mid-Century hybrid 'Harmony'	NW X Har	77
" " 'Ace'	X	" " "	A X Har	77
" " 'Nellie White'	X	'Mountaineer'	NW X M	77
" " 'Ace'	X	'Mountaineer'	A X M	77
" " 'Nellie White'	X	'Talisman'	NW X Tal	77
" " 'Ace'	X	"	A X Tal	77
" " 'Nellie White'	X	Lilium tigrinum open pollinated '66-12'	NW X 66-12	77
" " 'Ace'	X	" " " " "	A X 66-12	77
" " 'Nellie White'	X	Lilium martagon x L. hansonii	NW X MxH	77
" " " "	X	'Gift Bulb 66'	NW X G66	77
'Pink Perfection'	X	Lilium longiflorum 'Nellie White'	PP X NW	77
" "	X	" " 'Ace'	PP X A	77
'Mrs. Bangs hybrid'	X	" " 'Nellie White'	Mrs B X NW	77
" " "	X	" " 'Ace'	Mrs B X A	77
'Yellow Trumpet #3 x Yellow Trumpet #1'	X	" " 'Nellie White'	T3xT1 X NW	77
" " " " " " "	X	" " 'Ace'	T3xT1 X A	77
'Mrs. Bangs hybrid x Yellow Trumpet'	X	" " 'Nellie White'	66-10 X NW	77
'Gift Bulb 65 x Stiff Centifolium'	X	" " " "	66-9 X NW	77
Lilium henryi selfed x Gift Bulb 65	X	" " " "	66-8 X NW	77
" " " " " " "	X	" " 'Ace'	66-8 X A	77
'Orange Star Gift Bulb 61 x Yellow Trumpet'	X	" " "	61xYT X A	77
'Down-facing Yellow Trumpet x Outfacing Yellow Trumpet'	X	" " 'Nellie White'	DYTxOYT X NW	77
'Down-facing Yellow Trumpet x Outfacing Yellow Trumpet'	X	" " 'Ace'	DYTxOYT X A	77

TABLE 3. Results of the stylar-amputation stigmatic-exudate pollinations made in 1976, 1977 and 1978. 1977 and 1978 pollinations are also listed by type of cross.

YEAR	CROSS	NO. OF FLOWERS POLL.	NO. OF POLL. MADE	PODS WITH EMBRYOS	% PODS WITH EMBRYOS	NO. OF EMBRYOS	NO. OF EMBRYOS PER POD	% EMBRYOS PER POD	NO. OF PLANTS	NO. OF PODS WITH PLANTS
1976		2670	5736	7	.26	45	15.2	2.66	0	0
1977		971	2670	179	18.4	2810	15.2	5.66	120	37
	R X PG	2		1	50.0	15	15.0	5.0	0	0
	GS X PG	8		2	25.0	18	9.0	3.0	0	0
	ML X PG	2		1	50.0	6	6.0	2.0	0	0
	NW X PG	51		10	19.6	176	17.6	5.9	15	3
	A X PG	36		3	8.3	15	5.0	1.7	8	2
	NW X 65-1	48		11	22.9	128	11.6	3.9	5	4
	A X 65-1	25		2	8.0	18	9.0	3.0	0	0
	NW X 66-2	49		4	8.2	47	11.8	3.9	0	0
	A X 66-2	31		12	38.7	236	19.7	6.6	10	4
	NW X GB#8	48		4	8.3	38	9.5	3.2	0	0
	A X GB#8	29		3	10.3	14	4.7	1.6	0	0
	NW X YC#9	50		11	22.0	77	7.0	2.3	2	1
	NW X 7x9	69		13	18.8	127	9.8	3.3	4	3
	NW X 7x9 or X G66	17		1	5.9	33	33.0	11.0	0	0
	NW X G66	26		2	7.7	21	10.5	3.5	0	0
	NW X 67-1	97		9	9.3	89	9.9	3.3	1	1
	NW X Tal	44		6	13.6	68	11.3	3.8	3	1
	A X Tal	35		20	57.1	356	17.8	5.9	12	6
	NW X 66-12	50		29	58.0	489	16.8	5.6	12	4
	A X 66-12	31		26	83.9	751	28.9	9.6	12	6
	A X Har	33		5	15.2	28	5.6	1.9	0	0
	PP X NW	39		1	2.6	1	1.0	.3	0	0
	PP X A	41		4	9.8	59	14.8	4.9	29	2
	Others	110		0	0.0	0	0.0	0.0	0	0
1978		52	156	0	0.0	0	0.0	0.0	0	0
	NW X PG	30	90	0	0.0	0	0.0	0.0	0	0
	A X PG	22	66	0	0.0	0	0.0	0.0	0	0

TABLE Ia. Number of and type of pollinations made during the summer of 1977.

DATE	4/17	4/26	4/28	4/29	5/4	5/24	5/25	5/26	5/27	5/29	5/30	5/31	6/1	6/2	6/3	6/4	6/6	6/7	6/8	6/9	6/10	6/11	6/13	6/14	6/15	6/16	6/17	6/20	6/22	6/24	6/27	TOTAL
CROSS																																
GS X PG	1	3	2	1	1																											8
R9 X PG		2																														2
ML X PG		2																														2
NW X PG						15	9	3	5	3		8		7		1																51
A X PG												7	12	3	3		3		1		1											36
NW X 65-1							25	3	2	8		5		1		2	1		1													48
A X 65-1									3		5	6		6		2	2		1													25
NW X 65-2							18	3	3			17		4		2	1															46
A X 65#3							1		2		6				15	1	4															29
NW X TAL											19		14		1			10														44
A X TAL											15		14							34		1										35
NW X HAR															10			8														7
A X HAR																				29		3	1									33
NW X 66-12																			41		7		1		1							50
A X 66-12																				20		2	3		3		1	2				31
NW X 66-2									8		11		15		5		7		3													49
A X 66-2								7	1		3		7		5		5		3													31
NW X C#9																		40			7		3									50
NW X (7x9																			49		1		19									69
NW X MN											8		2					8														18
NW X MXM								2																								2
NW X G66																							7		6		6	3	1	2	1	25
NW X 67-1														29	32			30			6											91
Mrs 3 X NW														6																		6
Mrs 3 X A														7																		7
PP X NW																			1		8			11		10		9				39
PF X A																			1					17		10		11	2			41
NW X G66 or 7x9																					17											17
T3xT X NW																	1															1
T3xT XA																					1											1
66-9 X NW																										1				2		3
61xYT X A																												2	2	1		5
MAx66-10 X NW																												3	1			4
MCx66-10 X A																												3	2			5
DYTxDYT X NW																												2	3			5
DYTxDYT X A																														2		2
66-8 X NW																														2		2
66-8 X A																														2		2
TOTAL	1	7	2	1	1	15	53	18	24	11	67	43	64	61	77	11	27	96	101	83	48	6	34	28	10	21	7	39	11	11	1	971

TABLE 2a. Numerical list of interspecific hybrids obtained from 1977 pollinations. Pollination dates, excision dates and planting dates as well as intervals between these dates are given.

#	CROSS	ID NO	POLL DATE	EXCI DATE	INT PO-EX	PLANT DATE	INT EX-PL	INT PO-PL	STATUS
1	NW X PG	4-1							dead
2	NW X PG	4-08-2	5/25	7/12	48	8/12	31	79	dead
3	NW X PG	4-08-2	5/25	7/12	48	8/12	31	73	
4	NW X PG	4-07-	5/25	7/12	48	8/12	31	79	dead
5	NW X PG	4-07-	5/25	7/12	48	8/12	31	79	dead
6	NW X 65-1	6-11-8	5/27	7/13	47	8/22	40	87	
7	NW X PG	4-07-1	5/25	7/12	48	8/30	49	97	dead
8	NW X 67-1	42-09-10	6/10	7/15	35	9/1	48	83	dead
9	NW X PG	4-07-6	5/25	7/12	48	9/1	51	99	
10	A X PG	5-03-2	6/6	7/21	45	9/1	42	87	dead
11	A X 66-2	9-08-7	6/6	7/18	42	9/1	45	87	dead
12	NW X 65-1	6-07-3	5/25	7/13	49	9/1	50	99	dead
13	A X 66-2	9-04-5	5/27	7/15	49	9/1	48	97	dead
14	A X Tal	13-05-1	6/9	8/3	55	9/1	29	84	dead
15	A X Tal	13-02-1	6/9	7/25	46	9/1	38	84	dead
16	A X Tal	13-02-1	6/9	7/25	46	9/1	38	84	
17	A X 66-2	9-08-4	6/6	7/19	42	9/3	47	89	
18	A X 66-2	9-05-10	5/26	7/18	53	9/3	47	100	
19	A X 66-12	17-07-8	6/9	8/1	53	9/7	37	90	
20	A X 66-2	9-05-5	5/26	7/18	53	9/7	51	104	dead
21	NW X PG	4-07-3	5/25	7/12	48	9/7	57	105	
22	NW X PG	4-10-11	5/27	7/14	48	9/7	55	103	
23	A X PG	5-03-1	6/6	7/21	45	9/7	48	93	
24	A X Tal	13-08-1	6/9	8/5	57	9/13	39	96	
25	NW X PG	4-10-5	5/27	7/14	48	9/13	61	109	dead
26	A X 66-12	17-12-5	6/9	8/4	56	9/14	41	97	dead
27	A X 66-12	17-12-	6/9	8/4	56	9/14	41	97	
28	A X Tal	13-08-3	6/9	8/5	57	9/14	40	97	
29	A X 66-2	9-06-12	5/26	7/18	53	9/14	58	111	
30	NW X 65-1	6-07-2	5/25	7/13	49	9/16	65	114	dead
31	A X 66-12	17-19-3	6/9	8/9	61	9/16	38	99	
32	NW X 7-9	19-08-2	6/13	7/19	36	9/20	63	99	dead
33	NW X PG	4-07-3	5/25	7/12	48	9/20	70	118	dead
34	NW X PG	4-07-3	5/25	7/12	48	9/20	70	118	dead
35	NW X 7-9	19-08-2	6/13	7/19	36	9/20	63	99	dead
36	A X Tal	13-08-4	6/3	8/5	57	9/28	54	111	
37	A X PG	5-02-3	6/3	7/20	47	9/29	70	117	
38	PP X A	23-02-10	6/14	8/18	65	9/29	41	106	
39	A X PG	5-02-1	6/3	7/20	47	9/28	70	117	
40	NW X 66-12	16-08-11	6/8	7/26	48	9/28	64	112	
41	PP X A	23-02-6	6/14	8/18	65	9/24	37	102	
42	PP X A	23-02-6	6/14	8/18	65	9/24	37	102	
43	PP X A	23-02-6	6/14	8/18	65	9/24	37	102	
44	PP X A	23-02-4	6/14	8/18	65	9/24	37	102	
45	PP X A	23-02-4	6/14	8/18	65	9/24	37	102	
46	PP X A	23-02-4	6/14	8/18	65	9/24	37	102	
47	PP X A	23-05-3	6/14	8/22	69	9/24	33	102	
48	PP X A	23-05-3	6/14	8/22	69	9/24	33	102	
49	PP X A	23-02-1	6/14	8/18	69	9/24	37	102	dead
50	PP X A	23-02-1	6/14	8/18	65	9/24	37	102	
51	PP X A	23-02-1	6/14	8/18	69	9/24	37	102	
52	NW X 66-12	16-05-5	6/9	7/26	48	9/24	60	108	dead
53	PP X A	23-05-2	6/14	8/22	69	9/24	33	102	
54	PP X A	23-05-2	6/14	8/22	69	9/24	33	102	
55	PP X A	23-05-2	6/14	8/22	69	9/24	33	102	
56	A X 66-2	9-09-9	6/6	7/18	42	9/24	68	110	
57	PP X A	23-02-5	6/14	8/18	65	9/28	41	106	
58	PP X A	23-02-9	6/14	8/18	65	9/28	41	106	
59	NW X 7-9	19-09-2	6/13	7/27	44	9/28	63	107	
60	A X Tal	13-07-2	6/9	8/14	56	9/28	55	111	
61	A X Tal	13-04-1	6/9	8/3	55	9/28	56	111	
62	A X Tal	13-05-4	6/9	8/5	57	10/7	63	120	dead
63	A X Tal	13-09-6	6/9	8/5	57	10/7	63	120	
64	A X 66-12	17-09-16	6/9	8/2	54	10/14	73	127	
65	PP X A	23-02-3	6/14	8/19	65	10/14	57	122	dead
66	NW X 65-1	6-09-2	5/25	7/13	49	10/14	93	142	
67	PP X A	23-02-13	6/14	8/18	65	10/14	57	122	
68	A X 66-12	17-07-2	6/9	8/1	53	10/14	74	127	
69	A X 66-12	17-01-1	6/9	7/25	46	10/14	81	127	
70	PP X A	23-02-11	6/14	8/18	65	10/14	57	122	
71	PP X A	23-02-8	6/14	8/18	65	10/14	57	122	
72	PP X A	23-02-13	6/14	8/18	65	10/14	57	122	
73	A X Tal	13-07-2	6/9	8/4	56	10/17	74	130	
74	NW X 7-9	19-07-1	6/13	7/19	36	10/17	100	136	
75	A X 66-12	17-04-13	6/9	7/25	46	10/27	94	140	
76	PP X A	23-07-11	6/14	8/18	65	10/27	70	135	
77	PP X A	23-02-12	6/14	8/18	65	10/27	70	135	
78	PP X A	23-02-5	6/14	8/18	65	11/12	86	151	
79	PP X A	23-02-5	6/14	8/18	65	11/12	86	151	
80	PP X A	23-02-14	6/14	8/19	65	11/12	86	151	
81	PP X A	23-02-14	6/14	8/18	65	11/12	86	151	
82	NW X PG	4-07-4	5/25	7/12	48	11/12	123	171	
83	NW X Tal	12-07-4	6/7	8/3	57	11/14	103	160	dead
84	NW X Tal	12-07-4	6/7	8/1	57	11/14	103	160	
85	NW X Tal	12-07-2	6/7	8/3	57	11/14	103	160	
86	PP X A	23-02-12	6/14	8/18	65	12/28	132	197	
87	A X 66-2	9-08-6	6/6	7/18	42	12/28	163	205	
88	A X Tal	13-04-3	6/9	8/3	55	1/18	168	223	
89	A X 66-12	17-07-3	6/9	8/1	53	1/18	170	223	
90	A X 66-12	17-07-3	6/9	8/1	53	1/18	170	223	
91	NW X 66-12	16-09-4	6/9	7/26	48	1/18	176	224	
92	NW X 66-12	16-09-4	6/9	7/26	48	1/18	176	224	
93	NW X 66-12	16-09-4	6/9	7/26	48	1/18	176	224	
94	NW X 66-12	16-18-11	6/15	7/29	44	1/18	173	217	
95	NW X 66-12	16-18-11	6/15	7/29	44	1/18	173	217	
96	NW X PG	4-07-2	5/25	7/12	48	2/16	219	267	
97	NW X PG	4-07-2	5/25	7/12	48	2/16	219	267	
98	A X 66-12	17-03-4	6/9	8/1	53	2/16	199	252	
99	A X 66-12	17-07-4	6/9	8/1	53	2/16	199	252	
100	NW X YC99	18-07-4	6/13	7/18	35	2/16	213	248	
101	NW X YC99	18-07-4	6/13	7/18	35	2/16	213	248	dead
102	NW X 65-1	6-10-5	5/27	7/13	47	3/13	243	290	
103	A X 66-2	9-05-7	5/26	7/18	53	3/13	239	291	
104	NW X 66-12	16-09-4	6/8	7/26	48	4/4	252	300	
105	NW X 66-12	16-09-4	6/8	7/26	48	4/4	252	300	
106	NW X 66-12	16-09-4	6/8	7/26	48	4/4	252	300	
107	NW X 66-12	16-09-4	6/8	7/26	48	4/4	252	300	
108	NW X 66-12	16-09-4	6/8	7/26	48	4/4	252	300	
109	A X PG	5-02-3	6/3	7/20	47	4/4	258	305	
110	A X PG	5-02-3	6/3	7/20	47	4/4	258	305	
111	A X PG	5-02-3	6/3	7/20	47	4/4	258	305	
112	A X PG	5-02-3	6/3	7/20	47	4/4	258	305	
113	NW X 65-1	6-08-1	5/25	7/13	49	4/4	265	314	
114	A X 66-2	9-05-7	5/26	7/18	53	4/4	260	313	
115	NW X 66-12	16-18-11	6/15	7/29	44	1/18	173	217	
116	NW X 66-12	16-18-11	6/15	7/29	44	1/18	173	217	
117	NW X 66-12	16-18-11	6/15	7/29	44	1/18	173	217	
118	NW X 66-12	16-18-11	6/15	7/29	44	1/18	173	217	
					52.5		5d 5	151	

TABLE 2b. List of interspecific hybrids by parantage obtained from 1977 pollinations. Pollination dates, excision dates and planting dates as well as intervals between these dates are given.

#	CROSS	ID NO	POLL DATE	EXCI DATE	INT PO-EX	PLANT DATE	INT EX-PL	INT PO-PL	STATUS
4	NW X PG	4-07-	5/25	7/12	48	8/12	31	79	dead
5		4-07-	5/25	7/12	48	8/12	31	79	dead
7		4-07-1	5/25	7/12	48	8/30	49	97	dead
96		4-07-2	5/25	7/12	48	2/16	219	267	
97		4-07-2	5/25	7/12	48	2/16.	219	267	
21		4-07-3	5/25	7/12	48	9/7	57	105	
33		4-07-3	5/25	7/12	48	9/20	70	118	dead
34		4-07-3	5/25	7/12	48	9/20	70	118	dead
82		4-07-4	5/25	7/12	48	11/12	123	171	
9		4-07-6	5/25	7/12	48	9/1	51	99	
2		4-08-2	5/25	7/12	48	8/12	31	79	dead
3		4-08-2	5/25	7/12	48	8/12	31	79	dead
25		4-10-5	5/27	7/14	48	9/13	61	109	dead
22		4-10-11	5/27	7/14	48	9/7	55	103	
1						8/12			dead
15					48		78.4	126.4.	6
39	A X PG	5-02-1	6/3	7/20	47	9/28	70	117	
37		5-02-3	6/3	7/20	47	9/28	70	117	
109		5-02-3	6/3	7/20	47	4/4	258	305	
110		5-02-3	6/3	7/20	47	4/4	258	305	
111		5-02-3	6/3	7/20	47	4/4	258	305	
112		5-02-3	6/3	7/20	47	4/4	258	305	
23		5-03-1	6/6	7/21	45	9/7	48	93	
10		5-03-2	6/6	7/21	45	9/1	42	87	dead
8					46.5		157.8	204.3	7
30	NW X 65-1	6-07-2	5/25	7/13	49	9/14	65	114	dead
12		6-07-3	5/25	7/13	49	9/1	50	99	dead
113		6-08-1	5/25	7/13	49	4/4	265	314	
66		6-08-2	5/25	7/13	49	10/14	93	142	
102		6-10-5	5/27	7/13	47	3/13	243	290	
6		6-11-18	5/27	7/13	47	8/22	40	87	
6					48.5		126	174.3	4
13	A X 66-2	9-04-5	5/27	7/15	49	9/1	48	97	dead
20		9-05-5	5/26	7/18	53	9/7	51	104	dead
103		9-05-7	5/26	7/18	53	3/13	238	291	
114		9-05-7	5/26	7/18	53	4/4	260	313	
18		9-05-10	5/26	7/18	53	9/3	47	100	
29		9-06-12	5/26	7/18	53	9/14	58	111	
87		9-08-6	6/6	7/18	42	12/28	163	205	
11		9-08-7	6/6	7/18	42	9/1	45	87	
56		9-08-9	6/6	6/18	42	9/24	68	110	
17		9-08-4	6/6	7/18	42	9/3	47	89	
10					48.2		102.5	150.7	8

#	CROSS	ID NO	POLL DATE	EXCI DATE	INT PO-EX	PLANT DATE	INT EX-PL	INT PO-PL	STATUS
83	NW X Tal	12-07-4	6/7	8/3	57	11/14	103	160	dead
84		12-07-4	6/7	8/3	57	11/14	103	160	
85		12-07-2	6/7	8/3	57	11/14	103	160	
3					57		103	160	2
15	A X Tal	13-02-1	6/9	7/25	46	9/1	38	84	dead
16		13-02-1	6/9	7/25	46	9/1	38	84	
61		13-04-1	6/9	8/3	55	9/28	56	111	
88		13-04-3	6/9	8/3	55	1/18	168	223	
14		13-05-1	6/9	8/3	55	9/1	29	84	dead
60		13-07-2	6/9	8/14	56	9/28	55	111	
73		13-07-2	6/9	8/4	51	10/17	74	130	
24		13-08-1	6/9	8/5	57	9/13	39	96	
28		13-08-3	6/9	8/5	57	9/14	40	97	
36		13-08-4	6/9	8/5	57	9/28	54	111	
62		13-08-4	6/9	8/5	57	10/7	63	120	dead
63		13-09-6	6/9	8/5	57	10/7	63	120	
12					54.1		59.8	114.3	9
52	NW X 66-12	16-05 5	6/8	7/26	48	9/24	60	108	dead
40		16-08-11	6/8	7/26	48	9/28	64	112	
91		16-09-4	6/8	7/26	48	1/18	176	224	
92		16-09-4	6/8	7/26	48	1/18	176	224	
93		16-09-4	6/8	7/26	48	1/18	176	224	
104		16-09-4	6/8	7/26	48	4/4	252	300	
105		16-09-4	6/8	7/26	48	4/4	252	300	
106		16-09-4	6/8	7/26	48	4/4	252	300	
107		16-09-4	6/8	7/26	48	4/4	252	300	
108		16-09-4	6/8	7/26	48	4/4	252	300	
94		16-18-11	6/15	7/29	44	1/18	173	217	
95		16-18-11	6/15	7/29	44	1/18	173	217	
115		16-18-11	6/15	7/29	44	1/18	173	217	
116		16-18-11	6/15	7/29	44	1/18	173	217	
117		16-18-11	6/15	7/29	44	1/18	173	217	
118		16-18-11	6/15	7/29	44	1/18	173	217	
16					46.5		184.4	230.9	
69	A X 66-12	17-03-1	6/9	7/25	46	10/14	81	127	
98		17-03-4	6/9	8/1	53	2/16	199	252	
75		17-04-13	6/9	7/25	46	10/27	94	140	
68		17-07-2	6/9	8/1	53	10/14	74	127	
89		17-07-3	6/9	8/1	53	1/18	170	223	
90		17-07-3	6/9	8/1	53	1/18	170	223	
99		17-07-4	6/9	8/1	53	2/16	199	252	
19		17-07-12	6/9	8/1	53	9/7	37	90	dead
64		17-08-16	6/9	8/2	54	10/14	73	127	
26		17-12-5	6/9	8/4	56	9/14	41	97	dead
27		17-12-5	6/9	8/4	56	9/14	41	97	
31		17-18-3	6/9	8/9	61	9/16	38	99	
12					53.1		101.4	154.5	10

#	CROSS	ID NO	POLL DATE	EXCI DATE	INT PO-EX	PLANT DATE	INT EX-PL	INT PO-PL	STATUS
100	NW X YC#9	18-07-4	6/13	7/18	35	2/16	213	248	
101		18-07-4	6/13	7/18	35	2/16	213	248	dead
2					35		213	248	
74	NW X 7x9	19-07-1	6/13	7/19	36	10/27	100	136	
32		19-08-2	6/13	7/19	36	9/20	63	99	dead
35		19-08-2	6/13	7/19	36	9/20	63	99	dead
59		19-09-2	6/13	7/27	44	9/28	63	107	
4					38		72.3	110.3	
8	NW X 67-1	42-09-10	6/10	7/15	35	9/1	48	83	dead
1									
49	PP X A	23-02-1	6/14	8/18	65	9/24	37	102	dead
50		23-02-1	6/14	8/18	65	9/24	37	102	
51		23-02-1	6/14	8/18	65	9/24	37	102	
44		23-02-4	6/14	8/18	65	9/24	37	102	
45		23-02-4	6/14	8/18	65	9/24	37	102	
46		23-02-4	6/14	8/18	65	9/24	37	102	
78		23-02-5	6/14	8/18	65	11/12	86	151	
79		23-02-5	6/14	8/18	65	11/12	86	151	
41		23-02-6	6/14	8/18	65	9/24	37	102	
42		23-02-6	6/14	8/18	65	9/24	37	102	
43		23-02-6	6/14	8/18	65	9/24	37	102	
65		23-02-8	6/14	8/18	65	10/14	57	122	dead
71		23-02-8	6/14	8/18	65	10/14	57	122	
57		23-02-9	6/14	8/18	65	9/28	41	106	
58		23-02-9	6/14	8/18	65	9/28	41	106	
38		23-02-10	6/14	8/18	65	9/28	41	106	
70		23-02-11	6/14	8/18	65	10/14	57	122	
76		23-02-11	6/14	8/18	65	10/27	70	135	
77		23-02-12	6/14	8/18	65	10/27	70	135	
86		23-02-12	6/14	8/18	65	12/28	132	197	
67		23-02-13	6/14	8/18	65	10/14	57	122	
72		23-02-13	6/14	8/18	65	10/14	57	122	
80		23-02-14	6/14	8/18	65	11/12	86	151	
81		23-02-14	6/14	8/18	65	11/12	86	151	
53		23-04-2	6/14	8/22	69	9/24	33	102	
54		23-04-2	6/14	8/22	69	9/24	33	102	
55		23-04-2	6/14	8/22	69	9/24	33	102	
47		23-04-3	6/14	8/22	69	9/24	33	102	
48		23-04-3	6/14	8/22	69	9/24	33	102	
29					65.7		52.5	118.2	27

TABLE 3a. Procedure and chemicals needed to prepare Norstog's culture medium.

Label bottles with Norstog:Stock

- Add chemicals 1 at a time and don't add second one or next one until 1st or previous is clearly dissolved.

NORSTOG. 1973 In Vitro 8(4):307-308.

			Amount mg/liter	10 x/1 liter Weigh Out mg/liter
Stock I -	N:	Major minerals		
		KH_2PO_4	910	9100 mg
		KCl	750	7500 mg
		$MgSO_4 \cdot 7H_2O$	740	7400
		$CaCl_2 \cdot 2H_2O$	740	7400
Stock II -	N:	Trace elements:		100 x/500 ml
		$MnSO_4 \cdot H_2O$	3.0	300 mg
		H_3BO_3	0.5	50
		$ZnSO_4 \cdot 7H_2O$	0.5	50
		$CoCl_2 \cdot 6H_2O$	0.025	2.5
		$CuSO_4 \cdot 5H_2O$	0.025	2.5
		$NaMoO_4$	0.025	2.5
				100 x/500 ml
Stock III -	N:	Fe - Citrate	10.0	1000 mg
Stock IV -	N:	Vitamins		100 x/500 ml
		Inositol (meso)	50.0	5000 mg
		Thiamine - HCl	0.25	25
		Ca - pantothenate	0.25	25
		Pyridoxine - HCl	0.25	25
Stock V -	N:	Amino Acids		20 x/400 ml
		L-glutamine	400	8000 mg
		L-alanine	50	1000
		L-cysteine	20	400
		L-arginine	10	200
		L-leucine	10	200
		L-phenylalanine	10	200
		L-tyrosine	10	200

VI - MALIC ACID - Dissolve 1000 mg in 30 ml H_2O. Adjust pH to 5.0 with ml NH_4OH

Difco Purified Agar	6000 mg
Sucrose	34200 mg

Norstog: Mixing:

A) To 800 ml doubly-distilled H_2O (use deionized distilled) add 6 grams of Difco Purified Agar (7-9 gr if less pure Agar used) - Autoclave 15 min. @ 15 lb. pressure.

B) To ammonium malate solution (30 ml), add 100 ml major mineral stock (Stock I), 5 ml of trace elements (Stock II), 5 ml vitamins (Stock IV), 5 ml of Stock III (Fe-Citrate) + 20 ml of amino acids (Stock V), adjust pH to 4.9 with NaOH.
   - Add sucrose, (34.2 grams) + sufficient H_2O to yield 200 ml -
   - Filter through Millipore membrane and add to autoclaved component.

TABLE 3b. Procedure and chemicals needed to prepare Emsweller's culture medium.

Chemicals:	Stock I - E	mg/liter	Weigh Out 10 x/liter
	KH_2PO_4	200.0	2000 mg
	$MgSO_4 \cdot 7H_2O$	400.0	4000
	$Ca(NO_3)_2 \cdot 4H_2O$	800.0	8000
	KNO_3	200.0	2000
	Agar Difco Purified	6,000 mg	
	Sucrose	20,000 mg	

For 750 ml: To - 500 ml boiling water, add 4.5 grams Agar and 15 grams sucrose. Dissolve and add H_2O to 675 ml.

- Add 75 ml of Stock I. Mix thoroughly.

- Pour into vials - Plug - Autoclave 15 minutes.

INTERSPECIFIC HYBRIDIZATION OF *LILIUM LONGIFLORUM* THUNB.

by

DANIEL R. CLARK

B.S., Kansas State University, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

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Pre- and post- pollination applications of Easter lily stigmatic exudate on cut styles in stylar-amputation pollinations resulted in embryo formation and after embryo culture in plant development in 1977 pollinations. Stylar-amputation pollinations made in 1976 using only pre-pollination application of stigmatic exudate gave only a limited number of embryos and no plants. Pollinations were made using *Lilium longiflorum* or 1 of 4 Aurelian hybrids as female and crossing them with various Asiatic hybrid lilies.

Lilium longiflorum Thunb., the Easter lily, was crossed with numerous Asiatic hybrid lilies and Aurelian hybrid lilies. The pollination procedure involved cutting off at a 45° slant all but 1 cm of the style, applying Easter lily stigmatic exudate to the cut style surface, mixing pollen with the stigmatic exudate and reapplying stigmatic exudate. Flowers were repollinated twice by cutting off 3 mm of the remaining style, applying stigmatic exudate to the remaining stylar portion, and applying pollen and another drop of stigmatic exudate. Stigmatic exudate was used to carry pollen grains into the stylar canal. Embryos were excised from 30 to 70 days after pollination and ranged from small globular embryos to large torpedo shaped embryos. Approximately 120 hybrids with *Lilium longiflorum* have been obtained so far using these techniques.